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FINGERPRINTING *JUNIPERUS COMMUNIS* L. CULTIVARS USING RAPD MARKERS

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Abstract

Eight of eleven cultivars of *Juniperus communis* L. growing at Rancho Santa Ana Botanic Garden exhibit a morphology atypical of the wild populations from which they were reportedly derived. The "exotic" morphology consists of branchlets arranged at almost 90° to the branch axis and more spreading leaves that are of a bluer color than those of the wild plants. One of the "exotic" cultivars additionally shows a chimaeric distribution of acicular and scale-like leaves along its branches. Scale-like leaves are characteristics of *Juniperus* section *Sabina* and not section *Juniperus,* to which *J. communis* belongs. A RAPD marker study was initiated to compare RAPD fingerprints of the cultivars with those of their putative wild ancestors and representatives of other *Juniperus* species in both sections. Results suggested that the eight cultivars having an "exotic" morphology were either hybrids between *J. communis* and *J. chinensis,* or pedomorphic forms of *J. chinensis.* The three remaining cultivars that have a "native" morphology clustered with *J. communis* progenitors.

Rancho Santa Ana Botanic Garden (RSABG) is home to eleven plants of *Juniperus communis* L. (common juniper). All were established from cuttings reportedly taken from wild populations native to the California Floristic Province (Raven and Axelrod 1978). However, not all the plants in question exhibit the morphology generally seen in the wild. Instead of having a prostrate habit and somewhat incurved leaves, plants produce branches with fairly upright branchlets and spreading leaves. Given that RSABG specializes in the cultivation of plants native to California, a RAPD (Random Amplified Polymorphic DNA) marker study was initiated with the aim of tracing the origins of the putatively "exotic" specimens and to match up the remaining junipers with their wild progenitors.

Cultivars at Rancho Santa Ana Botanic Garden. Table 1 summarizes the salient characteristics and collection information of the junipers included in this study. Five of the eleven plants are of known geographic origin, but documentation for the remainder is either questionable or missing. Four distinctive morphologies are represented. The "exotic" form consists of long branches from which short branchlets emerge at an upward angle of almost 90°. Leaves are short and spreading and the entire plant is blue-green in color. This suite of traits is seen in CV1, CV3, CV4, CV6, and CV8–CV10. CV7 also differs by its greener foliage, a more spreading habit, and a chimaeric distribution of leaf shapes along its branches and branchlets. Zones of acicular, spreading leaves alternate with appressed, scale-like leaves reminiscent of species in section *Sabina.* The three remaining cultivars resemble *J. communis* found in the wild. CV2 has longer, incurved, leaves and a less prostrate habit with a moderately erect stem. A mat-like habit and incurved leaves characterize cultivars CV5 and CV11 (Table 1). The former is of greener and the latter of bluer coloring.

Juniperus communis *Varieties in the Western United States. Juniperus communis* is a circumboreal species of juniper (Franco 1962) characterized by acicular leaves. Two varieties of *J. communis* (Cronquist et al. 1972; Flora of North America Committee 1993) are encountered in the western United States. *Juniperus communis* var. *depressa* Pursh is native to the Great Basin Floristic Province. It ranges farther north into Alaska and eastward across much of Canada and the Great Lakes region, arching south along the east coast to North Carolina. *Juniperus communis* var. *montana* Aiton occurs from British Columbia southward into California in the Cascade Ranges, North Coast Ranges, and Sierra Nevada. The two varieties differ in habit, leaf size and shape and width of the glaucous stomatal band on the adaxial leaf surface. Although both are low-growing, variety *depressa* develops a somewhat erect main stem whereas variety *montana* is entirely prostrate. Leaf dimensions are ca. 1.0–1.6 mm broad × (6) 10–18 mm long (*depressa*), and (1.2) 1.5–1.8 mm broad × 5–10 (12) mm long (*montana*) (Cronquist et al. 1972). The glaucous stomatal band is as broad as, or narrower, than each green margin (*depressa*) or 2–3 times as broad as each green margin (*montana*). Two other varieties are occasionally distinguished in California. *Juniperus communis* var. *jackii* Rehder (Rehder 1940) differs from var. *montana* by having longer, more sparsely branched lateral branches. It is a form common to serpentinite substrates in inland coastal

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TABLE 1. DESIGNATION, ACCESSION NUMBER (WHERE KNOWN), LABEL OR PUTATIVE GEOGRAPHIC ORIGIN (WHERE KNOWN) AND SALIENT DISTINGUISHING CHARACTERISTICS OF CULTIVARS GROWING ON THE GROUNDS OF RANCHO SANTA ANA BOTANIC GARDEN.

Sample designation	Accession number	Label/putative geographic origin	Habitat and morphology
CV1	7106	CA, Mono County, 'Tioga'	fairly prostrate, long branches, branchlets upturned, leaves short & spreading, blue-green
CV2	9619	OR, Hood River County, Mt. Hood	erect main stem, long incurved leaves
CV3	13,360	OR, Curry County, 'Gold Beach'?	as CV1
CV4	?	?	as CV1
CV5	17,328	OR, Curry County, 'Gold Beach'	mat-like, long branches, leaves short & incurved, green; wild population no longer readily accessible
CV6	15,826	?	as CV1
CV7	15,826	?	branches spreading, some leaves scale-like, chimaeric distribution of acicular and scale-like leaves
CV8	?	?	as CV1, but foliage greener and branchlets inserted more evenly all around branch axis
CV9	7106	CA, Mono County, 'Tioga'	as CV1
CV10	7106	CA, Mono County, 'Tioga'	as CV1
CV11	17,329	CA, Del Norte County, 'Point St. George'?	mat-like, long branches, leaves short & incurved, blue-green; wild population extirpated

areas of northern California and Oregon. *Juniperus communis* var. *sibirica* Rydb. describes a very prostrate, almost mat-like, form found on coastal bluffs in the extreme northwest of California and southwestern Oregon, and at Ebbett's Pass in the Sierra Nevada. According to Roof (1973), this variety is characterized by leaves that are more incurved, making it less prickly to the touch than *J. communis* vars. *jackii* or *montana.*

RAPD analysis was chosen as a quick and relatively inexpensive means of getting a fingerprint of the genome of each plant which could then be compared against similar fingerprints generated from the native populations. This technique has been applied successfully to *Juniperus* in other studies (Adams and Demeke 1993) addressing affinities between species of *Juniperus.*

Methods

Plant Material. Plant material was gathered from all *J. communis* cultivars growing at RSABG and from seven wild populations growing at localities from which the original cultivar cuttings had reportedly been collected. In some cases, plants had been acquired from a nursery that had reportedly established its plants from wild-collected stock. Where the source populations were no longer alive or accessible (CV11 and CV5, respectively) adjacent populations were collected instead. Details of collecting locality and morphology of the native populations are summarized in Table 2. The seven wild-collected populations represent *J. communis* vars. *montana* (*mo*A–*mo*C, *mo*G) and *depressa* (*de*D–*de*F). Under the alternative taxonomic scheme (Table 2), populations *mo*A and *mo*G correspond to *J. communis* var. *sibirica,* and populations *mo*B and *mo*C to *J. communis* var. *jackii.* The fourteen cultivars added to the analysis after completion of the preliminary screens are identified in Table 3. They represent different cultivars of species of creeping juniper commonly sold in the nursery trade and are henceforth called "commercial" cultivars.

DNA Analysis. Leaf samples weighing 0.2–0.5 g were ground in liquid nitrogen, followed by extraction of genomic DNA using a modification of Doyle and Doyle (1987). Reaction mixtures (25 µl) for amplification of RAPD bands contained 0.1–1.0 µl genomic DNA (10 ng/µl), 18.8 µl dH_2O, 2.5 µl sequencing buffer (Tris-HCl (pH 9.0), KCl, $MgCl_2$, glycerin), 1.5 µl dNTP's (2.5 mM), 1.0 µl primer (10 pmol/µl), and 0.05 µl *Taq* polymerase. Details of primer nucleotide sequences are given in Table 4. Amplifications were performed on a PTC-100 thermocycler (MJ Research, Inc.) programmed for 1 cycle at 94°C for 1 min, 44 cycles at 94°C for 1 min, 42°C for 1 min and 72°C for 2 min, followed by a final extension time of 7 min at 72°C. Reaction product was run out on a 1.5% agarose gel, stained with ethidium bromide to visualize the bands, and electrophoregrams were photographed on a UV transilluminator (Fotodyne). Product size was determined using a DNA standard (1 kb ladder; Gibco BRL, Inc.). Bands were scored as present or absent by the first and last author. The scores were analyzed using the clustering algorithm UPGMA (Unweighted pair group method with arithmetic averages; average link) and Neighbor-Joining (NJ) available on PAUP* version 4.0 β1 (Swofford 1998).

Results

Of a total of 65 primers screened for RAPD analysis, six showed scorable and reproducible banding patterns and were entered into the final analysis. Scorable bands per primer ranged from one (Operon A1) to nine (UBC-244). A total of 34 bands were scored.

The preliminary analysis, which included all RSABG cultivars (CV), *J. communis* var. *depressa* (*de*) and all but the Ebbett's Pass population of *J. communis* var. *montana* (*mo*), revealed a strikingly different banding pattern of cultivars CV1, CV3, CV4, and CV6 through CV10. All had numerous bands that were missing from the three remaining cultivars and all wild populations. Clearly, the ancestry of these cultivars included an as yet unsampled genotype. The three cultivars having a set of bands more consistent with that of the wild populations were CV2, CV5 and CV11.

To identify the unknown parent or parental component, fourteen creeping cultivars of *J. chinensis* (3), *J. conferta* (1), *J. horizontalis* (6) and *J. sabina* (4) were added to the study (Table 3). Figures 1 and 2 show the resulting UPGMA and NJ phenograms. In both figures, the "exotic" and "commercial" cultivars were more similar to each other, forming a "non-native cluster", than to any of the wild populations ("native cluster"). Among the "commercial" cultivars, all *J. horizontalis* cultivars except *hor* 6 ('Wiltonii') formed a well-defined cluster, and another cluster contained all *J. sabina* cultivars, as well as *hor* 6. Perhaps *hor* 6 was mislabeled at the nursery of origin or has been mistakenly attributed to *J. horizontalis. Juniperus conferta* clustered with *J. sabina* (UPGMA; Fig. 1) or at the base of a cluster including the "exotics" and "commercial" cultivars (NJ; Fig. 2). *Juniperus chinensis* var. *sargentii* 'Viridis' (*chi* 3), did not cluster with the other two *J. chinensis* cultivars, regardless of the distance algorithm used. Instead, it clustered at the base of a cluster including the exotic RSABG cultivars, *chi* 1 and *chi* 2, and *J. horizontalis* (excluding *hor* 6).

Both clustering algorithms placed the "exotic" RSABG cultivars in a cluster with *J. chinensis* var. *procumbens* 'Nana' (*chi* 1) and *J. chinensis* 'San Jose' (*chi* 2). CV7 associated more closely with *chi* 1 and *chi* 2 than the other "exotics" in the NJ phenogram (Fig. 2). Even when *chi* 1 or *chi* 2 were excluded from the analysis the "exotics" still clustered with the "commercial" cultivars (not shown).

Only three RSABG cultivars clustered with the

TABLE 2. DESIGNATION, SCIENTIFIC NAME, COLLECTION LOCALITY, HABITAT AND SALIENT DISTINGUISHING FEATURES OF WILD POPULATIONS OF *JUNIPERUS COMMUNIS* VARIETIES. Nomenclature follows Flora of North America Committee 1993; names in parentheses are those used in Roof (1973). Sample designations consist of the first two letters of the varietal epithet (italic font), followed by the population (capital letter) and sample number, where more than one sample per population was obtained.

Sample designation	*J. communis* variety	Collection locality	Habitat notes	Habit and morphology
*mo*A1–*mo*A7	*montana* (sibirica)	OR, Curry County: Cape Sebastian	Coastal	mat-like, long branches, leaves short & incurved
*mo*B1–*mo*B6	*montana* (*jackii*)	CA, Del Norte County: Gasquet Toll Road; two sites ca. 1 mile apart	Near coast, serpentinite substrate	prostrate, very long branches
*mo*C1–*mo*C2	*montana* (*jackii*)	CA, Humboldt County: Onion Mountain/Onion Lake intersection	Near coast, serpentinite substrate	prostrate, fairly long branches (young plants)
*de*D	*depressa* (*saxatilis*)	UT, Iron County: between Cedar Breaks National Monument and Panguitch	Great Basin	erect main stem, leaves long & incurved
*de*E	*depressa* (*saxatilis*)	UT, Iron County: Cedar Breaks National Monument	Great Basin	erect main stem, leaves long & incurved
*de*F	*depressa* (*saxatilis*)	NV, White Pine County: Wheeler Mtn., Great Basin National Park	Great Basin	erect main stem, leaves long & incurved
*mo*G1–*mo*G3	*montana* (*sibirica*)	CA, Alpine County: Ebbett's Pass, Sierra Nevada	Sierra Nevada	mat-like, long branches, leaves short & incurved

TABLE 3. DESIGNATION, SPECIES AND CULTIVAR NAME, AND SECTIONAL PLACEMENT OF FOURTEEN "COMMERCIAL" CULTIVARS OF JUNIPER SPECIES ADDED TO THE STUDY TO TRACE THE PARENTAGE OF THE "EXOTIC" RSABG CULTIVARS.

Sample designation	Juniper species and cultivar name	Sectional placement
chi 1	*J. chinensis* var. *procumbens* 'Nana'	section *Sabina*
chi 2	*J. chinensis* 'San Jose'	section *Sabina*
chi 3	*J. chinensis* var. *sargentii* 'Viridis'	section *Sabina*
con 1	*J. conferta* 'Emerald Sea'	section *Juniperus*
hor 1	*J. horizontalis* 'Blue Chip'	section *Sabina*
hor 2	*J. horizontalis* 'Emerald Isle' ('Emerald Spreader'?)	section *Sabina*
hor 3	*J. horizontalis* 'Hughes'	section *Sabina*
hor 4	*J. horizontalis* 'Yukon Belle'	section *Sabina*
hor 5	*J. horizontalis* 'Prince of Wales'	section *Sabina*
hor 6	*J. horizontalis* 'Wiltonii'	section *Sabina*
sab 1	*J. sabina* 'Calgary Carpet'	section *Sabina*
sab 2	*J. sabina* 'Arcadia'	section *Sabina*
sab 3	*J. sabina* 'Moor-Dense'	section *Sabina*
sab 4	*J. sabina* 'Tamariscifolia'	section *Sabina*

native populations (Figs. 1 and 2). CV5 clustered with *mo*A1–*mo*A4 (Fig. 1) or *mo*B4 (Fig. 2). CV11 was placed in a cluster with *mo*A5 (Fig. 1) or with *mo*G1–*mo*G3 (Fig. 2). The long-leaved CV2 was less likely to group with any cluster. In the NJ phenogram (Fig. 2), it clustered with a four taxon cluster containing *mo*B3 and *de*D, *de*E and *de*F. However, UPGMA positioned CV2 at the base of the "native cluster" (Fig. 1). Results pertaining to the wild populations are discussed elsewhere (Ashworth et al., in prep.).

DISCUSSION

Phenetic analysis suggests that nine of eleven cultivars growing at RSABG are either similar to *J. chinensis* or are the result of hybridization between *J. communis* and *J. chinensis*. Given that all "exotics" showed banding patterns far more reminiscent of *J. chinensis* (*chi* 1 or *chi* 2) than their purported *J. communis* progenitor, rather than showing additivity, could reflect multiple backcrossing to the former (Hawkins and Harris 1998; Rieseberg and Ellstrand 1993). Variability within the wild populations makes it difficult to select among the *J. communis* varieties as the putative native ancestor. When all bands shared between the "exotics" and *chi* 1 or *chi* 2 are excluded from the cluster analysis, most "exotics" associate closest with *J. communis* var. *montana* population *mo*C1 (not shown). However, the large proportion of bands shared with *chi* 1 and especially *chi* 2 (73–85%) does not exclude the possibility of a pure *J. chinensis* origin. Under this scenario, the plants may represent pedomorphic *J. chinensis* mutants that retain acicular (juvenile) foliage instead of developing scale leaves typical of (mature) *J. chinensis*. Mutants are of common occurrence in *Juniperus* (p. 413, Flora of North America Committee 1993; Hall 1952).

If hybridity is invoked, the "exotics" may represent *J. communis* × *J. chinensis* hybrids that have undergone multiple backcrossing to *J. chinensis*. The NJ tree (Fig. 2) places CV7 closer to *chi* 1 and *chi* 2 than it does the other "exotics," possibly suggesting additional backcrossing events to *J. chinensis*, but this is not true of the UPGMA phenogram (Fig. 1). In the case of F_1 hybrids and morphological data, UPGMA has been shown to give more predictable placement of a hybrid with one or both parents than NJ (McDade 1997), but relative performances are unknown for more complex breeding histories, let alone for RAPD data and cases involving mutants. The placement of CV7 closest to *chi* 1 and *chi* 2 is, however, consistent with the observation that CV7 exhibits several *J. chinensis* characteristics, notably scale-like leaves and spreading branches, that are not found in the other "exotics". Overall, the RAPD data are in good agreement with morphology. All cultivars at RSABG suggested to be "exotic" by virtue of their less prostrate growth and more prickly leaves, display banding patterns atypical of the wild-collected plants while the cultivars of native appearance cluster with the wild populations. CV5 and CV11 exhibit the prostrate growth habit associated with native populations of *J. communis* var. *montana*. The blue-green foliage of CV11 and the green foliage of CV5 match

TABLE 4. NUCLEOTIDE SEQUENCES OF THE RAPD PRIMERS USED TO FINGERPRINT *JUNIPERUS* GENOTYPES IN THIS STUDY. All nucleotide sequences are cited in a 3′ to 5′ orientation.

Primer name	Nucleotide sequence
OPERON A1	CAG GCC CTT C
OPERON B18	CCA CAG CAG T
UBC-108	GTA TTG CCC T
UBC-111	AGT AGA CGG G
UBC-184	CAA ACG GCA C
UBC-244	CAG CCA ACC G
UBC-329	GCG AAC CTC C

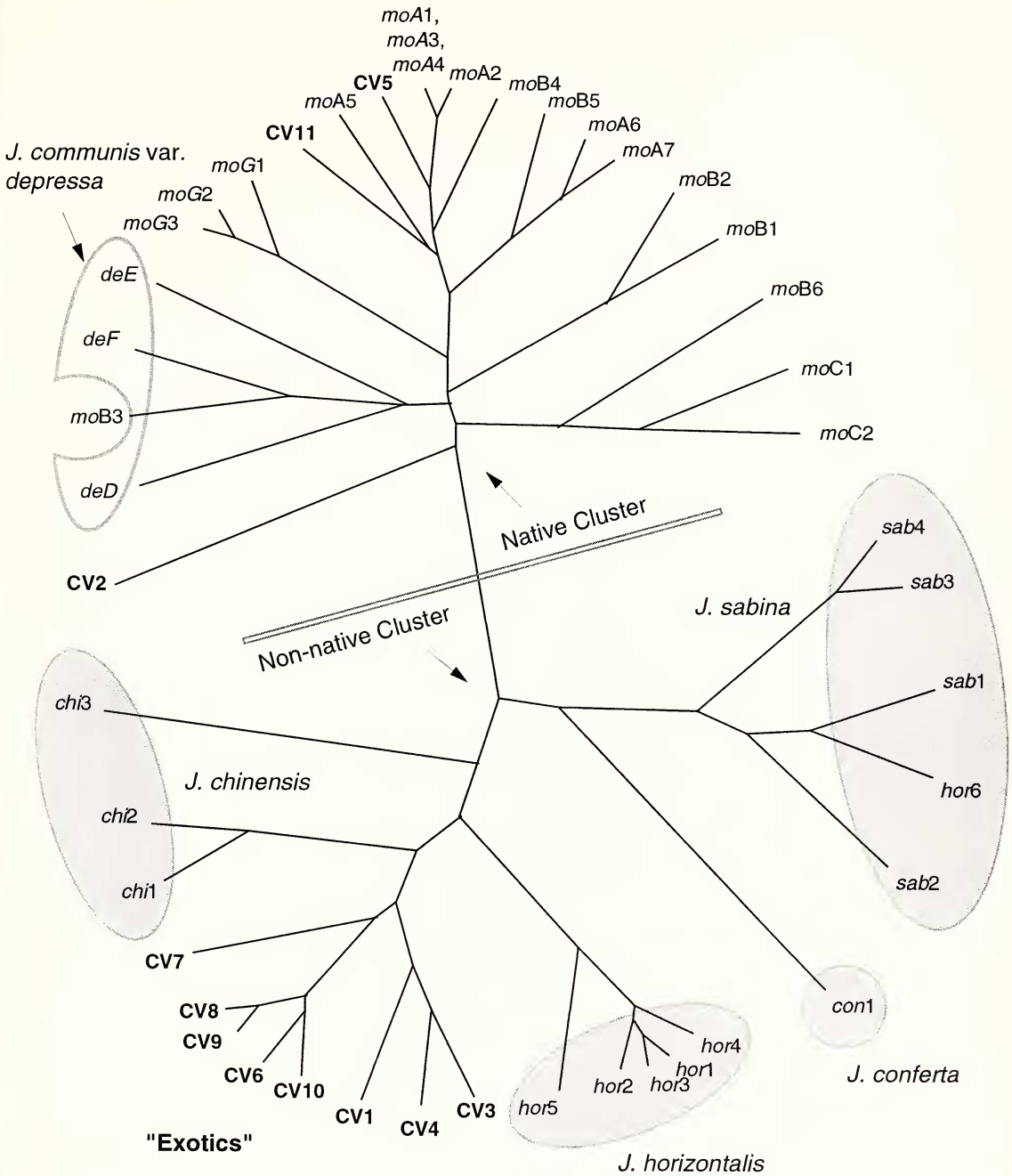

FIG. 1. Unrooted UPGMA phenogram, showing two distinct clusters that group plants with typical *Juniperus communis* morphology (native cluster) and "exotic" morphology (non-native cluster). All RSABG cultivars (CV1–CV11) are bolded. Shaded ovals highlight the four species of juniper other than *J. communis*. Members of *J. communis* var. *depressa* are also indicated. All other individuals represent *J. communis* var. *montana*. Sample designations are identical to those used in Tables 1–3.

Roof's (1973) description of the Point St. George and Gold Beach populations, respectively. CV2 resembles *J. communis* var. *depressa* in habit and leaf size, an affinity receiving partial support from the RAPD data (NJ; Fig. 2).

CONCLUSIONS

This study of dwarf junipers illustrates that a relatively simple molecular technique can be used to test a hypothesis based on observations of aberrant

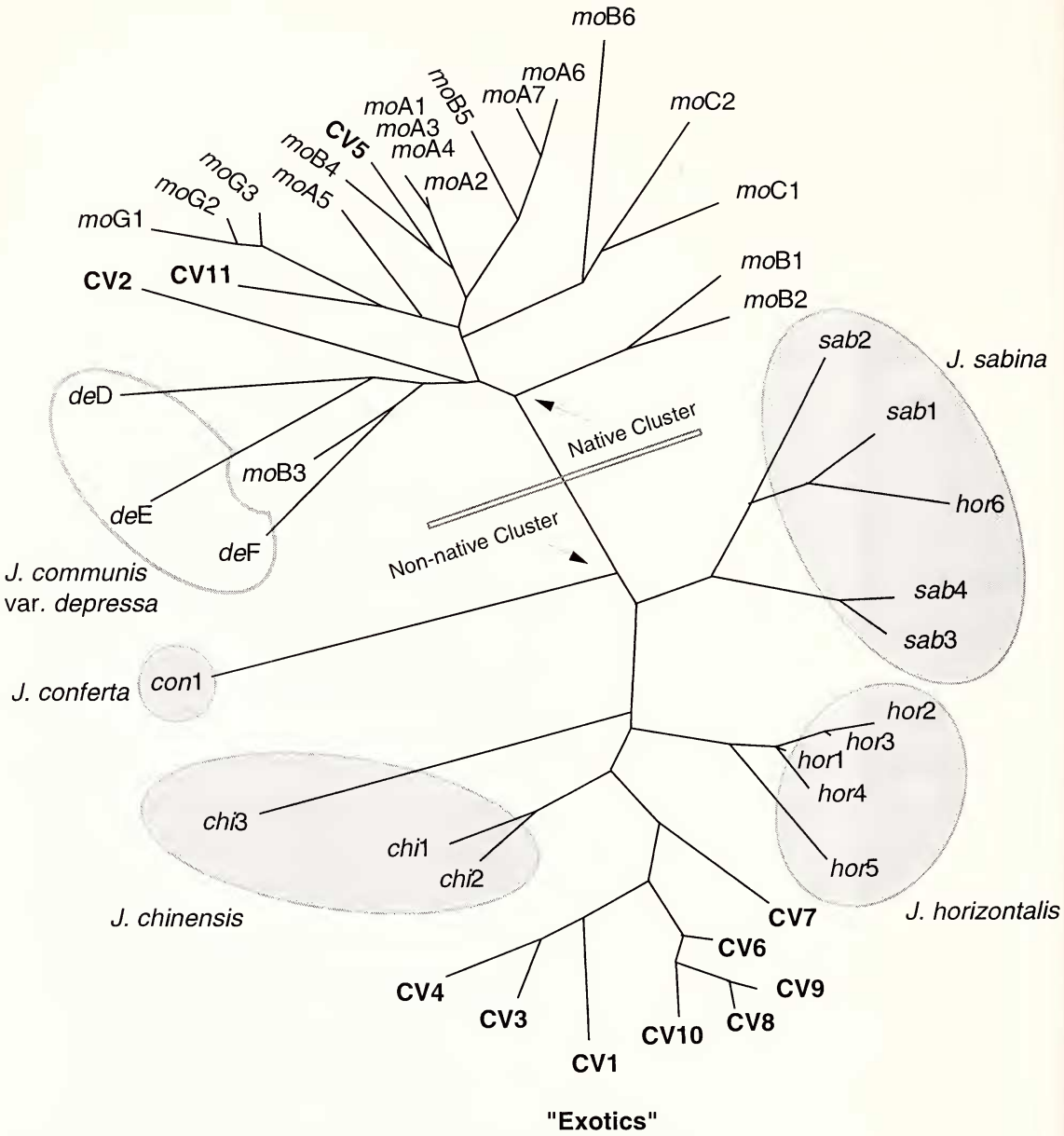

FIG. 2. Unrooted NJ phenogram. Abbreviations and explanations as in Figure 1.

plant morphology. Although the precise parentage of the "exotic" cultivars is unknown, the RAPD fingerprints nonetheless point to a major contribution from *J. chinensis*. Careful research into garden records suggests that all "exotics" trace back to three plants acquired from Louis L. Edmunds, Danville, CA, in 1950. These had been purportedly collected as cuttings from "just east of Tioga Pass summit" in 1938. The most likely explanation is a nursery mix-up, mislabeling, or inadvertent hybridization with *J. chinensis* (suggesting propagation from seed) in the intervening twelve years. It seems unlikely that the original plants from Tioga Pass were themselves hybrids or *J. chinensis* mutants, even though many species of this wind-pollinated genus are able to interbreed (e.g., Flora of North America Committee 1993) and *J. chinensis* has been in cultivation since the last century (Rehder 1940).

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